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Characterization of powdery mildew (*Leveillula taurica*) in globe artichoke (*Cynara scolymus*)

Enginarda (*Cynara scolymus*) külleme (*Leveillula taurica*) karakterizasyonu

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ABSTRACT

Globe artichoke (*Cynara scolymus* L.) is an economically important plant that is mostly grown in Mediterranean Basin in the world. In 2016 and 2017, chlorotic spots on adaxial surface but white fungal patches abaxial surface of leaves of globe artichoke were observed in the Western Mediterranean Region of Turkey. To determine causal agent of these symptoms, leaf samples were taken sporadically in 2016, 2017 and 2020. Based on morphological and ITS sequence data, the causal agent was determined to be powdery mildew fungus *Leveillula taurica* (Lév.) G. Arnaud. Pathogenicity test was conducted using seedlings of globe artichoke in a greenhouse. Additionally, infections caused by the fungus were examined in a two-year field study. Several surveys were carried out according to simple random sampling method and a total of 79 farmer fields (570 da) were screened in the region. Disease incidence and prevalence of the powdery mildew in 2016 were 14.2 and 36.1%, while they were 30.1 and 53.4% in 2017, respectively. Mean disease incidence and prevalence in the both years were 22.1 and 45.5%, respectively. The disease was previously reported on globe artichoke in Italy, U.S.A., Spain and Egypt. To our knowledge, this is the first record of *L. taurica* causing powdery mildew on globe artichoke in Turkey and globe artichoke is a new host for *L. taurica* in Turkey.

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ÖZ

Enginar dünyada çoğunlukla Akdeniz havzasında yetiştirilen ekonomik olarak önemli bir bitkidir. 2016 ve 2017 yıllarında Türkiye'de Batı Akdeniz Bölgesinde enginar bitkilerinin yapraklarının üst yüzeylerinde klorotik fakat alt yüzeylerinde beyaz fungal alanlar gözlenmiştir. Bu belirtilere neden olan etmeni tespit etmek amacıyla 2016, 2017 ve 2020 yıllarında belirli aralıklarla yaprak örnekleri alınmıştır. Morfolojik ve ITS sekans verilerine dayanarak etmenin külleme fungusu *Leveillula taurica* (Lév.) G. Arnaud olduğu belirlenmiştir. Patojenisite testleri enginar fideleri kullanılarak serada yürütülmüştür. Ayrıca, fungusun neden olduğu enfeksiyonlar iki yıllık bir arazi çalışması ile araştırılmıştır. Hastalık surveyleri basit tesadüfi örnekleme yöntemine göre yapılmış olup toplam 79 üretici tarlası (570 dekar) bölgede incelenmiştir. 2016 yılında hastalık oranı ve yaygınlığı sırasıyla %14.2 ve %36.1 olurken 2017 yılında bunlar sırasıyla %30.1 ve %53.4 olmuştur. Her iki yılda ortalama hastalık oranı ve yaygınlığı sırasıyla %22.1 ve %45.5 olmuştur. Hastalık daha önce İtalya, A.B.D., İspanya ve Mısır'da enginarda rapor edilmiştir. Bildiğimiz kadarıyla bu, Türkiye' de *L. taurica*'nın enginarda külleme hastalığının etmeni olduğuna dair ilk kayıt ve enginar *L. taurica* için Türkiye'de yeni bir konukçudur.

1. Introduction

Globe artichoke (*Cyanara scolymus*), belongs to *Asteraceae*, is an herbaceous plant domesticated in Roman times (Sonnante et al. 2007). It contains bioactive phenolic compounds, inulin, fibre, minerals and cynarin (1,3-O-dicaffeoylquinic acid). Therefore, it is a functional food source and one of the essential components of the Mediterranean cuisine (Lattanzio et al. 2009). Italy, Egypt and Spain are top producers of the crop with 389813, 323866 and 208463 t artichoke productions, respectively. With an annual 39477 t production, Turkey is 11th ranking place in the world (FAO 2020). However, the main threats to the production of globe artichoke are fungal diseases. In this regard, powdery mildews

cause considerable yield and quality losses in globe artichoke (Elsisi and Shams 2019).

The causal agent (*Leveillula taurica*) of powdery mildew of globe artichoke is an obligate fungus infecting a wide range of economically important crops such as pepper, tomato, eggplant, onion and cotton (Zheng et al. 2013a). For example, in South and North America, yield losses from *L. taurica* are frequently reported in tomato and potato production areas (Glawe et al. 2004; Sepúlveda-Chavera et al. 2013). Yield losses caused by *L. taurica* reachs up to 40% in pepper production in Puerto Rico (Negron et al. 1991). Infection rate of the fungus can reach 100% in pepper plants in Canada (Cerkauskas et al. 2011). Infections of *L. taurica* begin as small chlorotic spots on lower leaves in pepper plants. At first, these symptoms might be overlooked. However, ensuing colonisation, the fungus produces its conidia and conidiophores on abaxial surface of the infected leaves. Spreading these conidia initiates new infections on other non-infected plants (Zheng et al. 2013b).

Similar infections occur in other hosts of *L. taurica*. With regard to globe artichoke, little is known about infections and effects of *L. taurica* on production of the crop. Although it is stated that *L. taurica* is one of the major problems in globe artichoke production in California (U.S.A.), Italy, Spain, and Egypt (Snowdon 2010; Elsisi and Shams 2019). Little is known about powdery mildew fungus of globe artichoke. Occurrence of powdery mildew on globe artichoke was just reported without elaborating infections of *L. taurica* (Correll et al. 1987). Likewise, in Turkey, so far, there has been no study about it. The aims of the this study are i) reporting for the first time occurrence of powdery mildew on globe artichoke in Turkey, ii) characterising the causal agent of powdery mildew, and iii) examining incidence, prevalence and effects of the disease on globe artichoke production in the Western Mediterranean Region of Turkey.

2. Materials and Methods

2.1. Surveys

Surveys were conducted in eight locations (Muratpaşa, Kepez, Döşemealtı, Aksu, Serik, Manavgat, Gazipaşa and Kumluca) in Antalya province in the Western Mediterranean Region of Turkey in 2016 and in 2017. Simple random sampling method was used in the surveys (Bora and Karaca 1970; Aktaş 2001).

2.2. Identification of powdery mildew fungus

2.2.1. Morphological identification

At least 50 conidia and conidiophores were measured from each leaf sample. Measurements were performed using an Olympus BX43 microscope with SC100 digital colour camera.

2.2.2. Molecular identification

A total of four samples, designated as Gzp-2016, Gzp-2017, Srk-2017 and Kck-2020, were studied for molecular diagnose. Conidia on abaxial surface of the leaf samples were transferred into eppendorf tubes. DNA of the samples was extracted according to DNA purification protocol of Promega. After DNA extraction, rDNA fragments were amplified using primer pairs ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3') (White et al. 1990). Amplifications were conducted in a SimpliAmp Thermocycler (Applied Biosystems, USA) and consisted of 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, annealing temperature at 58.5ºC for 1 min, 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were separated in 2% agarose gels, stained with safe DNA dye and visualized under UV light. Sequence analysis was performed by GENOKS (Çankaya-Ankara). The ITS sequences of the isolates, Gzp-2016, Gzp-2017, Srk-2017 and Kck-2020, were deposited at GenBank with the accession numbers of KX709873, MF327257, MF327258 and MT573880, respectively.

In addition, to compare relatedness of our isolates with the other isolates in the genbank at NCBI, a phylogenetic tree was constructed using neighbour-joining method in MEGA version 7.0 program.

2.2.3. Scanning electron microscope (SEM) visualization

Conidia of *L. taurica* were taken from newly emerging white fungal patches underside of leaves of globe artichoke via cellophane. They were put to stubs and coated with a gold-palladium layer. Afterwards, they were viewed under scanning electron microscope (SEM) (ZEISS LEO 1430, Germany). This process was done in the Medicine Faculty of Akdeniz University. Surface patterns of primary and secondary conidia were examined.

2.3. Pathogenicity test

Pathogenicity test described by Kavak (2004) was modified and used in the present study. Nine-week-old artichoke seedlings were transplanted into pots (25×25 cm) in a greenhouse. Three seedlings were inoculated by dusting diseased leaves on water-misted leaf surfaces. Afterwards, they were covered with polyethylene bags. In the controls, no inoculation was done. Seedlings were kept at 25 to 30°C with 70 to 80% relative humidity. After three days, polyethylene bags were removed. Fifteen days after inoculation, disease symptoms occurred like those observed in the field in inoculated plants, while no disease symptoms were seen in the control plants.

2.4. Determination of disease incidence and prevalence of powdery mildew

Sampling was done by proceeding in the direction of diagonal lines of the each field surveyed. A total of 100 plants were randomly selected and evaluated in terms of peresence of powdery mildew. Disease incidence (DI) for each field surveyed was determined using formula below (Bora and Karaca 1970; Aktaş 2001).

DI (%)= Number of diseased plants ÷ Total number of the plants × 100

Disease prevalence (DP) in each field was detected according to formula below (Bora and Karaca 1970).

DP (%)= ∑ DI × Field area (da) ÷ Total field area investigated × 100

3. Results

3.1. *Characterization of L. taurica*

Morphological features of the fungus were described as: conidiophores were 177.3-271.1 × 4.7-7.0 µm, developing from

internal mycelium and emerging through stomata singly or in groups of one to five with dimorphic conidia (Figure 1).

Primary conidia were lanceolate, 43.3 to 67.0 × 17.6 to 27.1 µm, length/width ratio, 2.6-2.5; secondary conidia were ellipsoid to cylindrical, and measured 45.2 to 75.7 × 15.8 to 23.5 µm, length/width ratio, 3.2-3.0 (Table 1). These morphological features were very similar to *L. taurica* on globe artichoke identified by Correll et al. (1987).

The ITS sequences of the isolates, Gzp-2016, Gzp-2017, Srk-2017 and Kck-2020, were deposited at GenBank with the accession numbers of KX709873, MF327257, MF327258 and MT573880, respectively. The sequences of the isolates displayed a 99% homology with the isolates (e.g. KF703447, MT125857, GQ860947 and KU886148) of *Leveillula taurica* in the genbank (http://www.ncbi.nlm.nih.gov). Eventually, based on the morphological and molecular data, the fungus was identified as *Leveillula taurica* (Lev.) Arn. (Khodaparast et al. 2001; George and Fox 2014). One example showing alignment of the isolate Gzp-2016 (KX709873) with other *Leveillula taurica* isolates at NCBI was given in Figure 2.

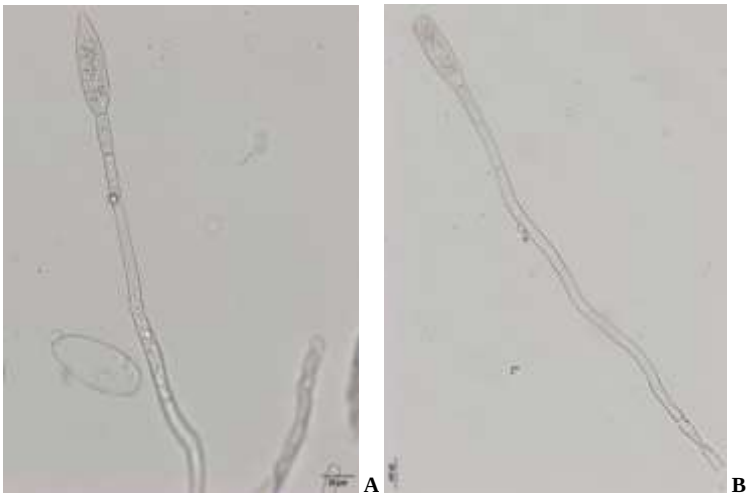
Surface of primary conidium was outcropping with wart like points. However, surface of secondary conidium was wrinkled with a net of oblong meshes covering almost all the surface area (Figure 3). These micromorphological features were pertained to *L. taurica* (Braun and Cook 2012).

The isolate (AB044991) of *L. taurica* was the closest match with our isolates in the phylogenetic tree (Figure 4).

3.2. Disease symptoms on globe artichoke in the field

Symptoms of powdery mildew on globe artichoke were as follows: yellowish spots with irregular margins were observed on the upper surfaces of the infected leaves, while grayish-white fungal masses were detected on the lower surface of those leaves (Figure 5).

These symptoms were initially observed on lower old leaves in globe artichoke plants. As the disease progressed, the similar symptoms were seen upper young leaves of the plants. Powdery mildew fungus (*L. taurica*) mostly caused leaf infections in globe artichoke. These symptoms are typical for the disease. However, apart from the leaves, in severe infections, disease symptoms occurred as white fungal patches on flower bracts of head of globe artichoke plants (Figure 6).



A **B**

Figure 1. Conidiophores bearing immature primary conidium (A) and secondary conidium (B) of *L. taurica* (scale bar= 50 µm).

Table 1. Morphological features of the isolates from different locations and years in globe artichoke growing felds of Antalya province.

Isolate code/ (accession numbers)	Conidiophores (length × width) (µm)	Mean	Primary conidia (length × width) (µm)	Mean	Length/ width ratio (µm)	Secondary conidia (length × width) (µm)	Mean	Length/ width ratio (µm)
Gzp-2016 (KX709873)	185.9 - 270.0 × 4.8 - 6.3	220.1 × 5.4	43.8 - 66.1 × 18.0 - 26.0	54.8 × 21.9	2.6	46.7 - 74.2 × 16.4 - 22.4	59.9 × 19.1	3.1
Gzp-2017 (MF327257)	192.7 - 259.4 × 5.1 - 6.5	225.6 × 5.8	44.0 - 65.5 × 17.6 - 26.6	55.2 × 22.5	2.5	47.0 - 75.7 × 17.2 - 22.6	60.8 × 19.8	3.0
Srk-2017 (MF327258)	197.4 - 271.1 × 5.0 - 6.7	234.3 × 5.9	43.3 - 66.7 × 17.8 - 27.0	55.7 × 22.0	2.6	46.4 - 75.1 × 17.8 - 23.0	60.5 × 19.4	3.1
Kck-2020 (MT573880)	177.3 - 264.9 × 4.7 - 7.0	237.9 × 5.2	43.9 - 67.0 × 17.7 - 27.1	54.9 × 22.2	2.5	45.2 - 74.4 × 15.8 - 23.5	59.6 × 19.0	3.2

Figure 2. The picture showing alignment of the isolate Gzp-2016 (KX709873) with other *Leveillula taurica* isolates at NCBI.

Figure 3. Surface patterns of primary (A) and secondary (B) conidia of *L. taurica*.

3.3. Incidence and prevalence of powdery mildew

Surveys of powdery mildew were carried out in eight locations (Muratpaşa, Kepez, Döşemealtı, Aksu, Serik, Manavgat, Gazipaşa and Kumluca) in Antalya province. In 2016, a 245 da artichoke growing area was surveyed, whereas in 2017 surveyed area was 325 da. Surveyed areas in both years constituted 34.8% of the total artichoke growing areas. Powdery mildew was observed in 36 farmer fields. In 2016, disease incidence was 14.2%, while it was 30.1% in 2017. Disease prevalence was 36.1% in 2016, whereas it was 53.4% in 2017. Disease incidence and prevalence were on average 22.1 and 45.5% in the both years, respectively (Table 2).

The first symptoms of the disease was established in April and infections continued until at the end of June. Meteorological data of this period (April-June) was given in Table 3.

4. Discussion

In the present study, fungal structures (conidiophores, primary and secondary conidia) of anamorphic stage of *Leveillula taurica* (Lév.) G. Arnaud were observed on diseased plants in the surveyed fields. However, no fungal formations of teleomorphic stage of the fungus were detected. Similarly, the teleomorphic stage of *L. taurica* was not observed on pepper (Cerkauskas et al. 2011) and caper plants (*Capparis spinosa*) plants in Italy (Bubici 2014). In addition, no teleomorphic stage of *L. taurica* was not detected on chickpea and *Cleome spinosa* plants in U.S.A. and Brazil (Attanayake et al. 2008; Carlos and Soares 2012). These findings indicate that without the need to form teleomorphic stage, *L. taurica* can maintain its survival by infecting other potential hosts surrounding all year round. This is probable for the Mediterranean region. Because, in this region, various agricultural crops including main hosts (e.g. tomatoa, potato, eggplant, cotton) of *L. taurica* are cultivated

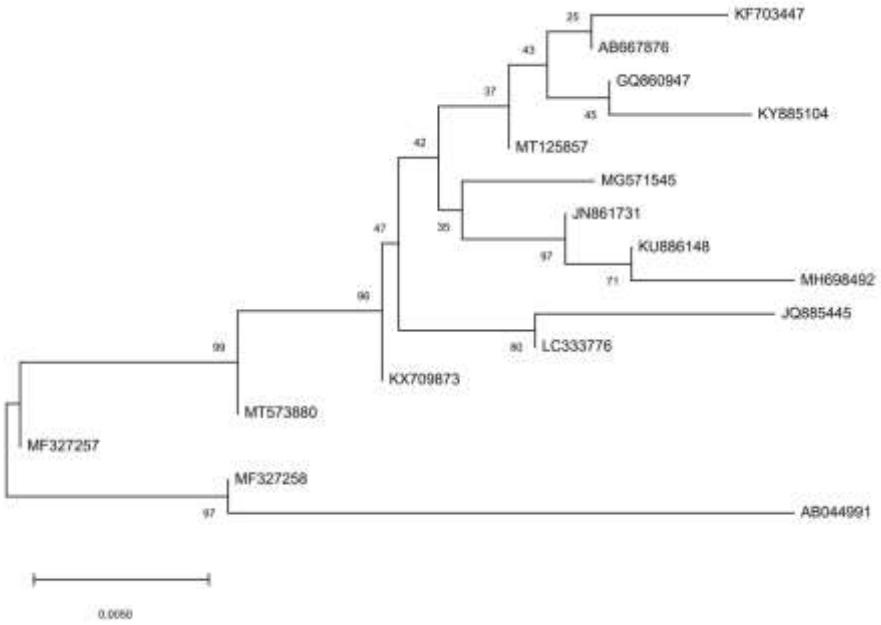


Figure 4. Phylogenetic tree constructed with *L. taurica* isolates displaying 99% homology with our isolates (KX709873, MF327257, MF327258 and MT573880).

A B

Figure 5. Yellow spots on adaxial surface (A) and white fungal masses (conidiophores and conidia) of *L. taurica* on abaxial surface of infected leaves of globe artichoke (B).

Figure 6. White powdery patches of *L. taurica* on flower bracts of globe artichoke.

Table 2. Artichoke growing areas of Antalya province, number of fields screened and areas surveyed in 2016 and 2017.

County	Cultivation area (da)		Locations	Surveyed area (da)		Number of surveyed field		Disease incidence (%)		Number of diseased fields /Total number of surveyed fields		Disease prevalence (%)	
	2016	2017		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Muratpaşa	10	5	Ermenek Topçular	10	5	2	2	12.4	28.0	1/2	1/2	50.0	50.0
Kepez	100	100	Gaziler	10	10	1	1	13.0	26.0	1/1	1/1	100.0	100.0
Döşemealtı	25	25	-	-	-	-	-	-	-	-	-	-	-
Aksu	255	255	Kundu Kurşunlu	10	10	2	1	12.0	26.0	1/2	1/1	50.0	100.0
Serik	600	600	Abdurrahmanlar Karadayı Yanköy Gebiz Çandır Karıncalı Tekkeköy	100	150	15	18	16.5	32.2	6/15	9/18	40.0	50.0
Manavgat	60	62	Cevizler Doğançam	-	25	-	5	-	34.5	-	2/5	-	40.0
Gazipaşa	380	345	Bakılar Ekmel Koru Pazarcı	65	75	12	12	15.4	30.2	3/12	7/12	25.0	58.3
Kumluca	50	50	Hasyurt Sarıcasu	50	50	4	4	16.0	34.0	1/4	2/4	25.0	50.0
Total	1490	1452		245	325	36	43	14.2	30.1	13/36	23/43	36.1	53.4
				570		79		22.1		36/79		45.5	

Table 3. Meteorological data of the locations surveyed in 2016 and 2017 (MEVBİS 2018).

Location	Months	Mean temperature (°C)		Mean relative humidity (%)		Total rainfall	
		2016	**2017**	**2016**	**2017**	**2016**	**2017**
Muratpaşa	April	19.1	17.7	68.1	62.3	14.4	46.8
Kepez	April	19.1	17.7	68.1	62.3	14.4	46.8
Aksu	April	18.2	16.4	68.7	69.3	10.4	28.8
Serik	April	19.3	17.0	59.6	61.0	12.0	69.0
Manavgat	April	19.3	17.2	65.1	66.3	0.0	75.7
Gazipaşa	April	18.5	16.9	62.6	61.0	12.8	48.2
Kumluca	April	17.6	15.7	69.1	70.2	31.3	27.0
Muratpaşa	May	20.4	21.3	72.9	67.7	28.2	38.5
Kepez	May	20.4	21.3	72.9	67.7	28.2	38.5
Aksu	May	20.3	20.5	67.1	73.9	15.6	35.2
Serik	May	20.7	21.1	63.1	64.1	26.7	86.4
Manavgat	May	20.8	21.3	68.6	69.4	0.0	60.6
Gazipaşa	May	20.4	20.5	64.0	64.3	61.2	55.0
Kumluca	May	19.8	19.5	66.3	71.9	6.6	32.8
Muratpaşa	June	26.9	26.3	62.8	63.1	24.5	3.3
Kepez	June	26.9	26.3	62.8	63.1	24.5	3.3
Aksu	June	26.1	25.6	61.6	66.9	18.2	0.0
Serik	June	27.4	26.3	51.8	59.4	15.1	3.6
Manavgat	June	27.2	26.1	57.2	65.3	0.0	0.0
Gazipaşa	June	26.3	24.9	54.4	61.1	0.0	0.0
Kumluca	June	25.2	24.5	58.3	62.7	0.6	0.0

year round. Moreover, apart from the agricultural crops, weeds may serve as a host for *L. taurica* as well. For example, field bindweed (*Convolvulus arvensis* L.) might be an inoculum source of *L. taurica* (Karkanis et al. 2012). In addition, climate of the Mediterranean prevailing in the region is extremely suitable for maintaing anamophic stage of the fungus. In fact, genus *Leveillula* is distributed in warm, arid areas of Africa, Asia, South America, southern Europe, and the western parts of

North America. Species within the genus are adapted to xerophytic conditions (Agrios 2005; Braun and Cook 2012). Considering all of these, it may be concluded that *L. taurica* may not form its teleomorphic stage if the conditions are favourable for the fungus.

In our study, there were variations in size of primary, secondary conidia and conidiophores of the isolates of *L. taurica*. Likewise, morphological variations of *L. taurica* were reported on various hosts (e.g. Jones et al. 2009; Glawe et al. 2010; He et al. 2012; Romberg et al. 2014; García-Gaytán et al. 2016; Choi et al. 2019). Moreover, Cerkauskas et al. (2011) reported significant morphological differences in size between *L. taurica* isolates from greenhouse-grown peppers and field-grown peppers. All of these imply that even in the same host plant, *L. taurica* may display morphological variations. However, in our study, morphology (primary and secondary conidia size) of our isolates was close to the measurements of *L. taurica* on globe artichoke reported by Correll et al. (1987). In addition, among the *L.* taurica isolates displaying a 99% homology with our isolates in the genbank, the closest one was the isolate (AB044991) of *L. taurica* from *Cirsium arvense* that is a perennial weed species of *Asteraceae*. These corroborated our finding of *L. taurica* isolates detected on globe artichoke (Asteraceae).

Unlike other powdery mildew fungus, *L. taurica* is endophytic nature advancing intercellularly and colonising mesophyll cells of leaf. As a result of this infection pattern, initially, chlorotic spots appear on adaxial surface of on leaves. But, the fungus forms its conidia and conidiophores on abaxial surface of the infected leaves. These fungal masses are seen as white fungal patches (Zheng et al. 2013b). Likewise, in our study, similar symptoms of powdery mildew occurred on leaves of globe artichoke. In addition to leaf infections, white fungal patches of *L. tauica* occurred on flower bracts of the globe artichoke plants in severe infections. Bratsch (2014) also reported that *L. taurica* could infect foliage of globe artichoke including flower bracts. In another study, *L. taurica* caused infections on leaves and stems of caper plant (*Capparis spinosa*) (Bubici 2014). In this regard, Agrios (2005) stated that apart from leaves, powdery mildew fungi could infect young shoots and stems, buds, flowers and young fruits. These may vary depending on powdery mildew fungus, host plant and environmental conditions. For example, in our study, powdery mildew symptoms aforementioned on flower bracts of globe artichoke occurred in severe infections in 2017.

Disease incidence and prevalance of powdery mildew were much higher in 2017 than 2016. This may have been related to the differences in environmental conditions of the both years. There were no distinct differences among mean temperatures of growing seasons in the both years (Table 3). However, mean relative humidity and in particular total rainfall in 2017 were considerably higher than 2016. This may have had an influence on the variations detected in disease incidence and prevalence of the both years. Because, relative humidity and wetness of foliage in plants are important environmental factors affecting infections of powdery mildew fungi. For example, in a study, relative humidity levels ranging from 20 to 40% decreased conidia germination of *L. taurica*. However, relative humidity levels varying from 50 to 70% increased conidia germination of the fungus (Guzman-Plazolaa et al. 2003). Favourable conditions for infection of *L. taurica* are mild temperatures ranging from 15 to 30˚C with relative humidity (60 to 100%) (Zayan 2016). Depending on these variables, disease infections in 2017 may have been more severe than 2016.

Powdery mildew fungi rarely kill their hosts but use their nutrients, reduce photosynthesis, increase respiration and transpiration. As a result, they cause disruption in plant growth and consequently decrease in yield up to 20 to 40% (Agrios 2005). In our study, considering disease incidence, mean of infection rate of *L. taurica* was 22.1%. This implies that *L. taurica* could cause considerable infection rates on globe artichoke in the Western Mediterranean region of Turkey. With regard to host, in a study, *L. taurica* was inoculated from pepper to various plant species to detect fungus's host range. As a result, different host responses ranging from highly susceptible to partially resistant were found. For example, globe artichoke was classified as highly susceptible host to *L. taurica* (de Souza and Café-Filho 2003). These findings indicate that *L. taurica* is not only a potential threat for globe artichoke but also for other crops.

In Turkey, *L. taurica* was reported on various crops such as sainfoin (Karakaya 1998), alfalfa (Eken and Demirci 2001), leek (Kurt et al. 2004), tomato (Ozan and Maden 2006), eggplant (Ozan and Aşkın 2006), hollyhock (Kavak and Dikilitas 2006). To our knowledge, globe artichoke is a new host for *L. taurica* in Turkey. In addition, the fungus was previously reported on globe artichoke in Italy, Morocco, Egypt, Israel, Spain and U.K. (Snowdon 2010). However, to our knowledge, this is the first record of *L. taurica* causing powdery mildew on globe artichoke in Turkey.

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